Transition metal-free reduction of activated alkenes using a living microorganism

Transition Metal-Free Reduction of Activated Alkenes using a Living Microorganism

Richard C. Brewster, Jack T. Suitor, Adam W. Bennett and Stephen Wallace*

Abstract: Microorganisms can be programmed to perform chemical synthesis via metabolic engineering. However, despite an increasing interest in the use of *de novo* metabolic pathways and designer whole-cells for small molecule synthesis, the inherent synthetic capabilities of native microorganisms remain underexplored. Herein we report the use of unmodified *E. coli* BL21(DE3) cells for the reduction of keto-acrylic compounds and apply this whole-cell biotransformation to the synthesis of aminolevulinic acid from a lignin-derived feedstock. The reduction reaction is rapid, chemo- and enantioselective, occurs under mild conditions (37 °C, aqueous media) and requires no toxic transition metals or external reductants. This study demonstrates the remarkable promiscuity of central metabolism in bacterial cells and how these processes can be leveraged for synthetic chemistry without the need for genetic manipulation.

Synthetic biology is revolutionizing the field of chemical synthesis. This approach uses recombinant DNA to enable the synthesis of a small molecule target directly from renewable feedstocks via fermentation.[1] Despite the elegance of this approach, microbial cells are frequently used in forward engineering endeavors as a biosynthetic starting-point, where synthetic capabilities are added via the introduction of new genetic parts. The background native chemical capabilities of these organisms remain underexplored. Research into the synthetic potential of native microbial cells could reveal new reactivity for use in synthetic chemistry and would also yield reactions that are inherently linked to cellular metabolism for use in metabolic pathway design. Furthermore, these reactions can also be interfaced with transition metal catalysis for the renewable synthesis of non-natural chemicals.[2] In a seminal example, Balskus et al. used H_2(g) generated via engineered metabolism in *E. coli* DD-2 to hydrogenate a range of alkene substrates using the biocompatible Royer Pd catalyst.[2d]

However, alkene reduction is also an inherent metabolic process of many native microorganisms. For example, facultative anaerobes use small organic molecules to respire via a reductive process. A classic example is the reduction of fumarate by fumarate reductase. This flavin-dependent C=C bond reduction can be used to support growth and replication under anaerobic conditions (Figure 1A). Other native enzymes can also be used to reduce C=C bonds. For example, *E. coli* BL21(DE3) and DH5α cells have been shown to reduce conjugated nitroalkenes in moderate yields and this has been attributed, in part, to the activity of the reductase NemA.[3] Furthermore, native Old Yellow Enzymes in the yeast *Saccharomyces cerevisiae* have also been used to reduce β-substituted, α,β-unsaturated carbonyl compounds in a stereospecific manner.[4]

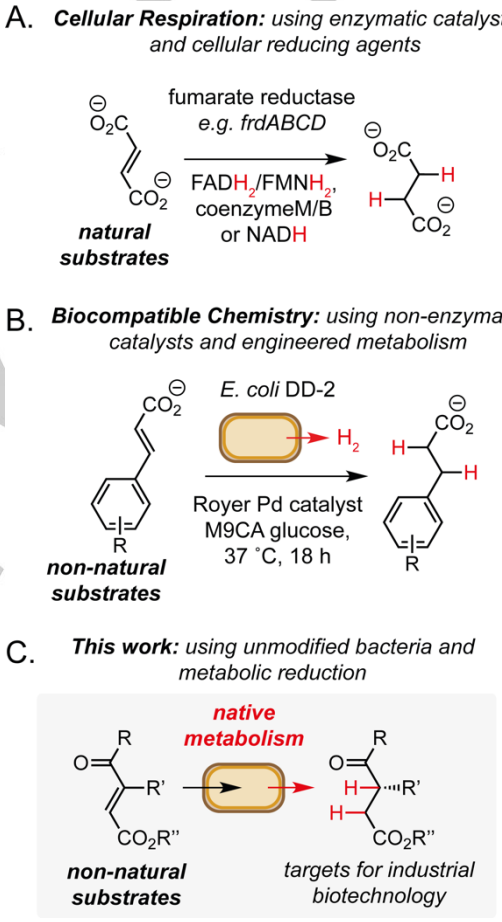

Figure 1. Cellular approaches to alkene reduction. A) Native respiratory enzymes reduce activated alkenes under anaerobic conditions. B) Metabolic H_2(g) production can be interfaced with a biocompatible Pd catalyst for the reduction of coumaric acids. C) Exploring the native reductive capability of unmodified microorganisms for transition metal-free keto-acrylate reduction.

Inspired by these studies, we set out to investigate the reduction of substituted keto-acrylic compounds (KACs) by microbial cells (Figure 1C). KACs feature in a range of pharmaceutical and fine chemical syntheses and are predominantly reduced in organic solvent using transition metals under an atmosphere of H_2(g). Biocatalytic approaches to KAC reduction have been limited to *in vitro* studies using purified enzymes. In 2014, Pietruszka *et al.* reported the use of the enoate reductase YqjM from *Bacillus subtilis* for the reduction of short-chain KACs *in vitro* and coupled this with an alcohol dehydrogenase (ADH_{LK}) to synthesize chiral γ-butyrolactones.[5] Despite the elegance of this approach, the

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strict cofactor dependence of YqjM and ADH_{LK} requires the use of exogenous NADPH and FMN, which must also be regenerated *in situ* using additional enzymes. The reaction also requires constant buffering using a pH-stat. There are currently no methods to achieve this reaction using green synthetic methods or microbial whole cells.

We reasoned that the similarity of the KAC motif to fumarate suggested that native microbial cells could facilitate this transformation without the need for genetic manipulation. This hypothesis was inspired by our previous studies in biocompatible chemistry where reduction of the unnatural compound diethyl maleate was observed during a cyclopropanation reaction and attributed to the metabolic activity of *E. coli* NST74.[2b] Furthermore, *E. coli* possesses a series of YqjM homologues in its genome ($\leq$33% identity, Table S10) and can generate reducing equivalents *in situ* via oxidative glucose metabolism. Therefore, we set out to investigate whether keto-acrylate reduction could be performed by living *E. coli* cells and, if so, whether this could be used in the synthesis of an industrially-relevant small molecule. Herein we report the discovery that the common laboratory strain of bacteria *E. coli* BL21(DE3) can reduce a range of keto-acrylates without the need for genetic modification. This whole-cell reaction is mild, efficient, enantioselective and represents an operationally simple and green alternative to current transition metal-based methods that utilize H_2(g).

We began our studies by investigating whether *E. coli* could reduce butenyl-substituted keto-acrylate esters **1** in cell culture (Figure 2A). The butenyl side chain was included to assess the chemoselectivity of any observed reduction. The benzyl ester **1**-Bn was included to localize the substrate to the cell membrane, and the carboxylic acid **1**-H was used as a hydrophilic control. Substrates were synthesized in 4 steps from commercially available glyoxal dimethyl acetal via a Horner-Wadsworth-Emmons olefination, acetal hydrolysis, Grignard addition and oxidation (see S2.1). The laboratory strain *E. coli* BL21(DE3) was chosen due to its widespread availability in molecular biology labs and its well-annotated genome. We were pleased to find that C=C reduction occurred for all the compounds tested, as confirmed by ^{1}H NMR (Figure 2B, C). The methyl and ethyl esters **1**-Me and **1**-Et were reduced in 39% and 43% yield, respectively, and were isolated alongside significant quantities of hydrolyzed substrate and product (Table S1). Hydrolysis was reduced to 8% using the bulkier benzyl ester **1**-Bn, which increased the product yield to 69%. However, use of the carboxylic acid substrate **1**-H eliminated this side-reaction and provided the desired reduced product **2**-H in quantitative yield (Figure 2C).

To determine whether the reaction was biocompatible we analyzed its effect on *E. coli* cells (Figure 2D and Table S2). The methyl and ethyl esters **1**-Me/**1**-Et were highly toxic and the benzyl ester reduced cell viability by 400-fold (Figure 2D, columns 2-4). The most biocompatible substrate was the carboxylic acid **1**-H, which caused only a 2-fold drop in cell viability after 48 h (Figure 2D, column 5). This observation suggested that the toxicity of **1**-Me/**1**-Et may be due to the release of MeOH/EtOH into the cell. However, when we cultured cells in the presence of **1**-H and an equimolar volume of EtOH, no toxicity was observed. Instead, cell viability increased by 3-fold (P<0.001, Figure 2D, column 6 and Table S8). The addition of EtOH is known to mimic the heat shock response in *E. coli* and increases the rigidity of the cell membrane, an effect that could account for this observation.[6] At this point, due to poor product conversions, competing hydrolysis and the high toxicity of reactions involving **1**-Me and **1**-Et we decided to focus the remainder of this study on the benzyl ester and carboxylic acid **1**-Bn and **1**-H.

Next, we tested the reaction at higher substrate concentration and investigated the rate of product formation during the reaction. Increasing the concentration of carboxylic acid **1**-H to 5 mM (ca. 0.8 g/L) decreased the yield of the reaction to 66% and reduced cell density by 77% (Figure 3A, columns 2-4). Interestingly, using 5 mM (ca. 1.2 g/L) of the benzyl ester **1**-Bn increased the reaction yield to 79% and increased the cell density to 118% of control samples containing no substrate (Figure 3A, columns 5-7).

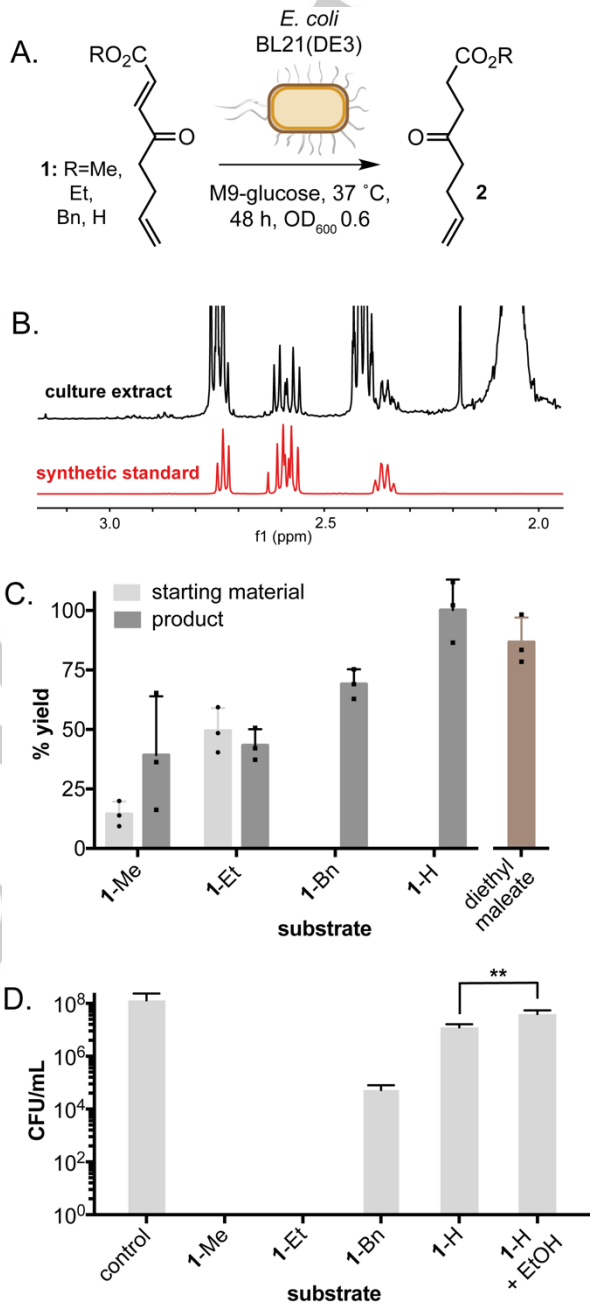

Figure 2. A) Initial KAC ester and carboxylic acid screen. B) Product identification via comparison of culture extracts with a synthetic standard by ^{1}H-NMR. C) Product yields for various substrate esters and carboxylic acid. D) Serial dilution and plate count assays. All substrates were added at 1 mM concentration. Reactions were performed in sealed Hungate tubes under an atmosphere of air for 48 h. *E. coli* BL21(DE3) cells transformed with an empty pEdinBrick plasmid (pSB1A2 derived, OD_{600}=0.6) were used to ensure the presence of a monoculture. All cultures were grown in M9 media containing D-glucose (4 g/L) and ampicillin (100 mg/L). Product concentrations in crude culture extracts were determined by ^{1}H NMR spectroscopy relative to an internal standard of 1,3,5-trimethoxybenzene. All data is shown as an average of three independent experiments to one standard deviation. **P<0.005

Differences between **1**-H and **1**-Bn were also observed when analyzing the rate of product formation over time. Firstly, we found that both reactions were complete in <6 h. Reduction of **1**-H was complete after 3 h, whereas the reduction of **1**-Bn was rapid during the first 1.5 h (65% conversion) and then plateaued before

reaching completion after 6 h (Figure 3B and Table S3). One explanation for this involves consideration of the effect(s) and relative location of **1**-Bn/**1**-H in the cell interior. Hydrophobic compounds are known to partition in the membrane of *E. coli* ($logP_{octanol/water}$ = 3.7 for **1**-Bn vs –1.7 for **1**-H at pH 7.4, Table S7) and this in-turn has been shown to accelerate glucose consumption via increased growth/maintenance demands.[7,8]

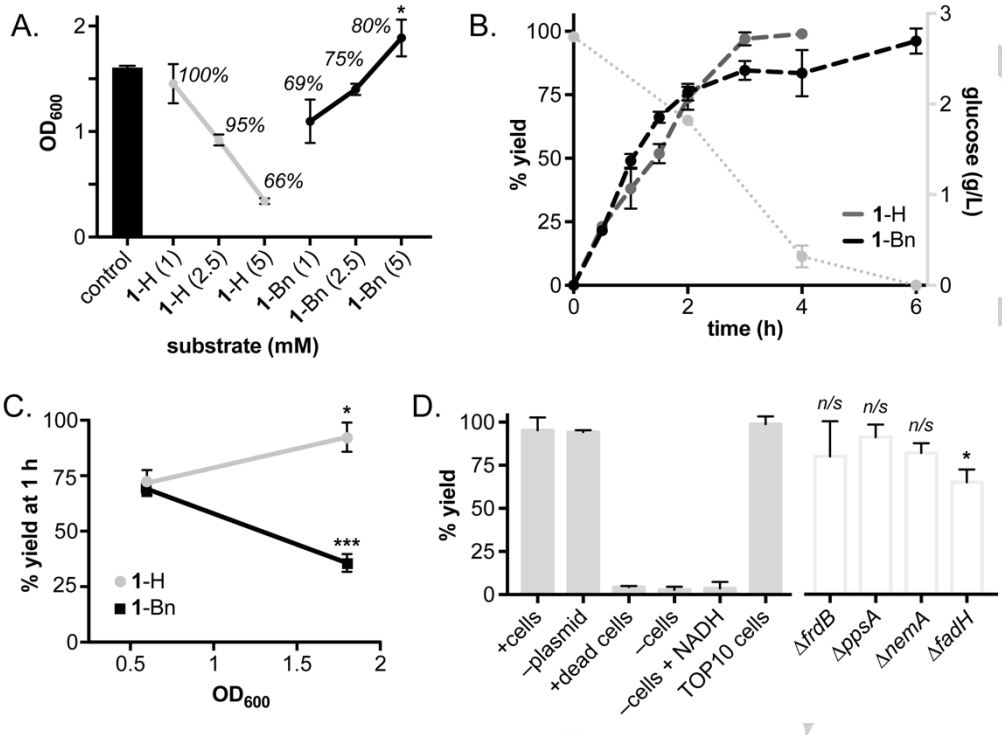

Figure 3. Investigating the effect of the reaction on *E. coli*. A) The effect of increasing substrate concentration on culture density and product yield. B) Time-course analysis of product formation and glucose levels during the reaction. C) The effect of varying cell density on the initial rate of product formation using suspended whole-cells. D) Control experiments investigating the effect of the components of the reaction on the reduction of **1**-H. Numbers indicated within the graphs represent the yield of the reaction. Substrates were added at 1 mM unless stated otherwise. All data is shown as an average of three independent experiments to one standard deviation. *P<0.05, ***P<0.0005, *n/s* = P>0.05

Indeed, we found that a three-fold increase in cell density accelerated the reduction of **1**-H after 1 h (72% yield at OD_{600}=0.6 vs. 92% yield at OD_{600}=1.8, *P*<0.05) but decreased the conversion of **1**-Bn (69% yield at OD_{600}=0.6 vs. 36% yield at OD_{600}=1.8, *P*<0.0005) (Figure 3C and Table S6). Furthermore, a prolonged elongation during cell division as a result of membrane stress can also increase the number of viable but non-culturable (VBNC) cells via *rpoS*-mediated pathways leading to higher cell density and reduced cell viability (Figure 3A and Table S9).[9] Together, these observations support the hypothesis that the decreased reduction of **1**-Bn after 1.5 h results from the cumulative effects of an altered cell physiology leading to depleted glucose levels (Figure 3B).

We next examined the effect of the reaction components on the reduction of **1**-H. Product formation was reduced to <5% yield in the absence of cells or the presence of dead cells (heated to 95 ˚C for 15 min). No reduction was observed in blank media, spent supernatant or in media containing excess NADH (10 equiv., 24 h). Conversely, **1**-H was reduced in >98% yield using *E. coli* TOP10 or DH5α cells, and in 94% yield using BL21(DE3) cells containing no pEdinBrick plasmid (Figure 3D and Table S11). Together, this data supports the hypothesis that **1** is reduced in the cell by an enzyme that is native to *E. coli*. To explore this further we examined the reaction using the knock-out strains *E. coli* JW4114(K-12)Δ*frdB*, JW1642(K-12)Δ*nemA*, JW3052(K-12)Δ*fadH* and JW1692-2Δ*ppsA*. *FrdB* encodes for the catalytic subunit of fumarate reductase, whereas *nemA*, *fadH* and *ppsA* encode for a *N*-ethylmaleimide reductase, 2,4-dienoyl-CoA reductase and phosphoenolpyruvate synthase, respectively. NemA, FadH and PpsA are homologs of the known KAC-reductase YqjM from *B. subtillus* (Table S12). To our surprise, the reduction of **1**-H was unaffected by the absence of the *frdB*, *nemA* or *ppsA* genes (77% ± 21%, 82% ± 4.6% and 91% ± 7.3% yield, respectively, P>0.05 relative to the parent Keio strain), whereas deletion of *fadH* reduced the product yield to 65% ± 6.0% (P<0.05, Figure 3D and Table S11). This partial yet significant effect has been observed whilst studying the reduction of nitroalkenes by *E. coli* BL21Δ*nemA*.[3] Together, this suggests the presence of either a multifaceted KAC reduction pathway in the cell interior or a potentially novel C=C keto-acrylate reductase in the genome of *Escherichia sp*.

Having shown that KAC reduction occurs rapidly under mild conditions using *E. coli* BL21(DE3) cells we next investigated the enantioselectivity of this process using α,β-substituted substrates. We synthesized the α- or β-methyl substituted carboxylic acid and benzyl ester substrates **3**-H-αMe, **3**-H-βMe, **3**-Bn-αMe and **3**-Bn-βMe from either glyoxal dimethyl acetal or pyruvic aldehyde dimethyl acetal in a similar manner to **1**-Bn/H (see S2.1). Under our optimized conditions, the α-methylated carboxylic acid **3**-H-αMe was reduced in 79% and the corresponding benzyl ester **3**-Bn-αMe was reduced in 98% yield. Conversely, the β-methylated isomers were both reduced in <5% yield (Figure 4A). Furthermore, both the α- and β-methyl benzyl ester substrates reduced cell viability by 1,500-fold, whereas the carboxylic acid congeners were biocompatible (Table S10). Analysis of the product of the reaction by chiral HPLC confirmed that the reduction of **3**-Bn-αMe was enantioselective, affording the reduced product in 87% e.r. The carboxylic acid **3**-H-αMe was reduced in 51% e.r. A cyclic α-substituted KAC substrate **6** was also reduced under the reaction conditions, affording the corresponding chiral cyclohexanone in 44% yield and 62% e.r. Moving the benzyl group to the α-position in **7** abolished any enantioselectivity. The simplified KAC substrate **5** was reduced in 100% yield and 77% e.r, whereas its *Z*-isomer was reduced in 69% yield and 79% e.r. In contrast, dimethyl maleate **8**-*Z* was reduced in 78% yield, whereas dimethyl fumarate **8**-*E* was reduced in 3% yield. Citraconate and itaconate di-esters (**9** and Figure S3) and acrylate **10** were unreactive under the reaction conditions.

Having confirmed the enantioselectivity of the reduction we moved on to apply the reaction to the synthesis of a target small molecule. Aminolevulinic acid (ALA, Levulan®, **13**) is a naturally-occurring non-proteogenic amino acid that is used as an additive in photodynamic therapy during the treatment of Paget's disease and cancer.[10] Although access to ALA via engineered metabolism has been achieved, this has not translated into a viable industrial bio-process.[11] ALA is therefore currently produced on an industrial scale via the oxidation of petrochemically-derived furfurylamine followed by a Pd-catalyzed hydrogenation using H_2(g). We reasoned that our *E. coli*-

mediated C=C reduction could achieve this same transformation without the need for toxic Pd salts or pressurized H_2(g). To this end, we synthesized the butenolide hemiacetal **12** from lignin-derived furan imide **11**. Upon addition to *E. coli* BL21(DE3) cells, **12** underwent ring-opening and C=C reduction, affording the reduced product in 81% isolated yield. Finally, the phthalimide was removed under acidic conditions to afford ALA in quantitative yield (Figure 4B).

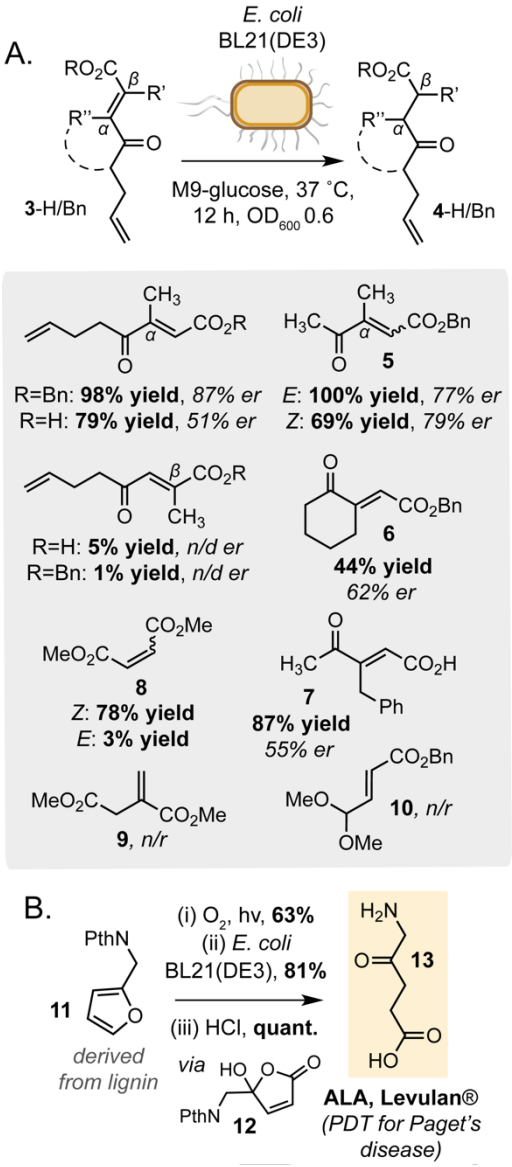

Figure 4. Investigating the chemo- and enantioselectivity of the whole-cell reaction and target-oriented synthesis. A) Asymmetric reduction of α- and β-methyl substituted KAC substrates. Yields are for reduction of the substrates shown. B) Synthesis of aminolevulinic acid from a lignin-derived furan imide derivative using an *E. coli* mediated alkene reduction as an alternative to Pd/C and H_2(g). Reactions were performed in sealed Hungate tubes under an atmosphere of air. Substrates were added at 1 mM. Cultures were grown in M9 media containing D-glucose (4 g/L) and ampicillin (100 mg/L). *n/r* = no reaction.

In general, the use of microbial cells is an operationally simple and green approach to fine chemical synthesis. In the case of this KAC reduction, *E. coli* BL21(DE3) cells are readily available, easily cultured, and possess all the cofactors and associated regeneration enzymes required to carry out C=C bond reduction. This avoids the use expensive exogenous reagents and circumvents the need for large-scale enzyme overexpression and purification, as purified enzymes must be continuously replenished via re-synthesis in the same way as a chemical reagent. The mild reaction conditions and absence of any transition metals also adds to the appeal of this process. This stands in stark contrast to modern abiotic methods for transition metal-free alkene reduction in synthetic organic chemistry, recent examples of which include: (i) transfer hydrogenation using diphenylamine, triethylsilane and the electrophilic cationic phosphonium catalyst $[(C_6F_5)_3PF)]^+[B(C_6F_5)_4]^-$, thiourea organocatalysis using a Hantzsch ester in refluxing H_2O and (ii) the use of $NaAlH_4$/C nanocomposites and 100 bar H_2(g) in organic solvent at 150 ˚C for 48 h.[12] The biocompatibility of whole-cell reactions is also important, yet is rarely considered in the field of biocatalysis. Beyond substrate localization effects, biocompatible reactions can also be readily integrated into *de novo* metabolic pathways for renewable chemical production in microbial cells.

In summary, we have shown that unmodified laboratory strains of *E. coli* can be used for the reduction of keto-acrylic acids, esters and α-substituted variants thereof. The reaction is efficient, occurs under mild conditions and uses no toxic transition metals or external reductants. The remarkable biocompatibility of this process led us to discover unique facets of the reaction in a cellular setting that will be used to inform the design of new whole-cell reactions. This includes, to the best of our knowledge, the first observation that reactivity effects vary when substrates are localized to different regions of the cell. Finally, we applied this reaction to the synthesis of the valuable small molecule aminolevulinic acid, eliminating the requirement for the use of Pd/C and H_2(g). Future studies will focus on identifying the enzyme(s) responsible for this reaction using comparative transcript-/proteomics and integration of this novel biotransformation into metabolic pathways for renewable small molecule synthesis via fermentation.

Acknowledgements

This work was supported by the University of Edinburgh and an ISSF grant from the Wellcome Trust. J.T.S. acknowledges a PhD Scholarship from the Carnegie Trust. We acknowledge Mr Alan Taylor and the Scottish Instrumentation and Resource Centre for Advanced Mass Spectrometry (SIRCAMS) for their assistance during the preparation of this manuscript.

Keywords: biotransformation • green chemistry • reduction • whole cell • biotechnology

[1] a) J. D. Keasling, *Science* **2010**, *330*, 1355-1358; b) J. Becker, C. Wittermann, *Angew. Chem. Int. Ed.* **2015**, *54*, 3328-3350; *Angew. Chem.* **2015**, *127*, 3383-3407; c) J. M. Clomburg, A. M. Crumbley, R. Gonzalez, *Science* **2017**, *355*, (6320).

[2] a) S. Wallace, E. P Balskus, *Curr. Opin. Biotechnol.* **2014**, *30*, 1-8; b) S. Wallace, E. P. Balskus, *Angew. Chem. Int. Ed.* **2015**, *54*, 7106-7109; c) S. Wallace, E. P Balskus, *Angew. Chem. Int. Ed.* **2016**, *55*, 6023-6027; d) G. Sirasani, L. Tong, E. P. Balskus, *Angew. Chem. Int. Ed.* **2014**, *53*, 7785-7788.

[3] P. Jovanovic, S. Jeremic, L. Dijokic, V. Savic, J. Radivojevic, V. Maslak, B. Ivkovic, B. Vasiljevic, J. Nikodinovic-Runic, *Enzyme Microb. Technol.* **2014**, *60*, 16-23.

[4] a) N. G. Turrini, R. C. Cioc, D. J. H. van der Niet, E. Ruijter, R. V. A. Orru, M. Hall, K. Faber, *Green Chem.* **2017**, *19*, 511-518; b) C. K. Winkler, K. Faber, M. Hall, *Curr. Opin. Chem. Biol.* **2018**, *43*, 97-105; c) E. Brenna, F. G. Gatti, D. Monti, F. Parmeggiani, A. Sacchetti, J. Valoti, *J. Mol. Catal., B Enzym.* **2015**, *114*, 77-85; d) C. K. Winkler, G. Tasnádi, D. Clay, M. Hall, K. Faber, *J. Biotechnol.* **2012**, *162*, 381-389; e) K. Durchschein, S. Wallner, P. Macheroux, W. Schwab, T. Winkler, W. Kreis, K. Faber, *Eur. J. Org. Chem.* **2012**, *26*, 4963-4968; f) E. Burda, T. Reß, T. Winkler, C. Giese, X. Kostrov, T. Huber, W. Hummel, H. Gröger, *Angew. Chem. Int. Ed.* **2013**, *52*, 9323-9326; g) H. S. Toogood, J. M. Gardiner, N. S.

Scrutton, *ChemCatChem* **2010**, *2*, 892-914; h) F. Hollmann, I. W. C. E. Arends, D. Holtmann, *Green Chem.* **2011**, *13*, 2285-2314.

[5] T. Classen, M. Korpak, M. Schölzel, J. Pietruszka, *ACS Catal.* **2014**, *4*, 1321-1331.

[6] K. M. Dombek, L. O. Ingram, *J. Bacteriol.* **1984**, *157(1)*, 233-239.

[7] a) J. Sikkema, J. A. de Bont, B. Poolman, *J. Biol. Chem.* **1994**, *269*, 8022-8028; b) A. Wahl, L. My, R. Dumoulin, J. N. Sturgis, E. Bouveret, *Mol. Microbiol.* **2011**, *80*, 1260-1275.

[8] *Chemicalize* was used for the prediction of logP values, June 2019, https://chemicalize.com/, developed by ChemAxon Ltd.

[9] a) M. Boaretti, M. del Mar Lleo, B. Bonato, C. Signoretto, P. Canepari, *Environ. Microbiol.* **2003**, *5*, 986-996; b) D. E. Bohannon, N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, R. Kolter, *J. Bacteriol.* **1991**, *173*, 4482-4492; c) R. Lange, R. Hengge-Aronis, *J. Bacteriol*. **1991**, *173*, 4474-4481; M. H. Rau, P. Calero, R. M. Lennen, K. S. Long, A. T. Nielsen, *Microb. Cell Fact.* **2016**, *15*:176; d) C. U. Chukwudi, L. Good, *Microb. Pathog.* **2018**, *114*, 393-401.

[10] a) K. Mikasa, D. Watanabe, C. Kondo, M. Kobayashi, H. Nakaseko, K. Yokoo, Y. Tamada, Y. Matsumoto, *J. Dermatol.* **2005**, *32*, 97-101; E. V. Filonenko, A. D. Kaprin, B. Y. A. Alekseev, O. I. Apolikhin, E. K. Slovohodov, V. I. Ivanova-Radkevich, A. N. Urlova, *Photodiagnosis Photodyn. Ther.* **2016**, *16*, 106-109; c) X. Yang, P. Palasubernaim, D. Kraus, B. Chen*, Int. J. Mol. Sci.* **2015**, *16*, 25865-25880; d) B. Nokes, M. Apel, C. Jones, G. Brown, J. E. Lang, *J. Surg. Res.* **2013**, *181*, 262-271; e) A. A. Nardelli, T. Stafinski, D. Menon, *BMC Dermatology*, **2011**, 11:13.

[11] a) Z. Kang, Y. Wang, P. Gu, Q. Wang, Q. Qi, *Metab. Eng.* **2011**, *13*, 492-498; b) T. Li, Y-Y. Guo, G-Q. Qiao, G-Q. Chen, *ACS Synth. Biol.* **2016**, *5*, 1264-1274.

[12] a) M. Pérez, C. B. Caputo, R. Dobrovetsky, D. S. Stephan, *Proc. Natl. Acad. Sci. USA*, **2014**, *111*, 10917-10921; b) G. Weng, X. Ma, D. Fang, P. Tan, L. Wang, L. Yang, Y. Zhang, S. Qian, Z. Wang, *RSC Adv.* **2017**, *7*, 22909-22912; c) P. L. Bramwell, J. Gao, B. de Waal, K. P. de Jong, R. J. M. Klein Gebbink, P. E. de Jongh, *J. Catal.* **2016**, *344*, 129-135.

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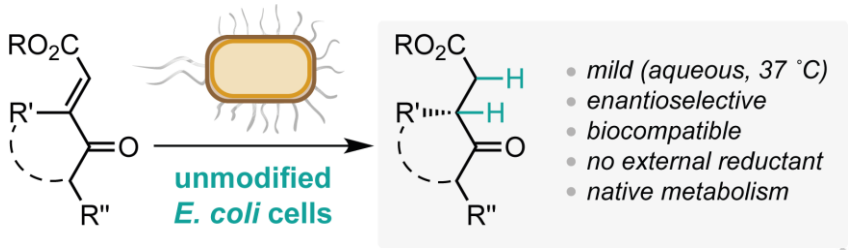

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Alkene-Reducing Bugs: The microorganism *Escherichia coli* BL21(DE3) was found to reduce a range of keto-acrylic alkene substrates without the need for genetic modification. The reaction is mild, enantioselective and provides a green alternative to transition metal-based methods that utilize H_2(g). The biocompatibility of the reaction was examined in a cellular setting and used to inform the metal-free synthesis of aminolevulinic acid from a lignin-derived substrate.